

SOIL BACTERIA AND GROWTH-PROMOTING SUBSTANCES¹

A. G. LOCHHEAD

Bacteriology Division, Science Service, Canada Department of Agriculture, Ottawa, Canada

CONTENTS

I. Introduction.....	145
II. Growth Factors in Soil.....	145
III. Synthetic and Destructive Processes.....	147
IV. Vitamin-Requiring Bacteria in Soil.....	147
A. Basic Procedures.....	147
B. Bacteria Requiring Vitamin B ₁₂	147
C. Bacteria Requiring the Terregens Factor.....	148
D. Specific Vitamin Requirements of the Predominant Bacterial Flora.....	148
V. Synthesis of Growth Factors by Soil Bacteria.....	149
A. Capability of the Predominant Soil Bacteria for Vitamin Synthesis.....	149
VI. Discussion.....	150
A. Some Analogies between Soil and Aquatic Environments.....	150
B. Growth-Promoting Substances and Soil Problems.....	151
VII. References.....	151

I. INTRODUCTION

Microbial growth-promoting substances in soil and organisms requiring or synthesizing them have received less attention than the subject of antagonisms. Growth factors, however, may be considered in the same category with antibiotics insofar as they affect the development of many microorganisms at very low concentrations and may be produced by microbial synthesis. In view, therefore, of the highly diversified nutritional requirements of the soil microflora, it is reasonable to state that growth factor effects could well be as important as antibiotic activity in affecting the microbial equilibrium in such a densely populated environment as arable soil.

It is now generally conceded that most, if not all, of the B-vitamins, as well as other microbial growth factors, are present in fertile soil. Their occurrence has been ascribed to various processes: to release from vitamin-containing plant and animal residues, including organic fertilizers, to liberation from the roots of growing plants, and probably most important, to synthesis by microorganisms.

Although it had been previously recognized that soil extract exerted a favorable effect on the growth of many bacteria and other microorganisms, it is largely within the past two decades that it has been possible to distinguish effects ascribable, for example, to readily avail-

able nitrogen or improved mineral supplies from those provided by growth factors *per se*.

II. GROWTH FACTORS IN SOIL

Most of the earlier studies on growth-promoting substances in soil were concerned with the stimulation of plant growth, a subject beyond the scope of this review. However, in 1920 Mockridge (28) reported that "auximones" extracted from decomposed composts may stimulate not only plants, but also the activity of soil organisms; the production of the stimulating substances was ascribed to microbial synthesis although their nature remained largely obscure. Of like interest is the work of Sanborn (37) who reported that aqueous extracts of soil in which crop material had undergone decomposition markedly stimulated a cellulose-decomposing organism, *Cellulomonas folia*, both in growth and physiological efficiency. In 1935 Pringsheim (32) observed that an extract of soil was important to the development of several species of flagellates and suggested that a growth-stimulating substance of the "bios" group was involved. The same year Lwoff and Lederer (27) found that an extract of soil stimulated markedly the growth of the flagellate *Polytomella agilis* and concluded that the effect was attributable, not to humic substances or the mineral elements of the extract, but to growth factors.

The occurrence of specific vitamins in soil seems to have been first demonstrated by Lilly

¹ Contribution No. 446, Bacteriology Division.

and Leonian (15) in 1939. By the use of selected species of fungi requiring, respectively, the complete thiamin molecule (*Phytophthora erythroseptica*), both components, thiazole and pyrimidine (*Phycomyces blakesleeanus*), and the single components (*Mucor ramannianus* and *Pythiormorpha gonapodioides*), they showed that growth could, in all cases, be induced by additions of extracts of soils. The presence of biotin was also deduced by the ability of a soil extract to permit growth of *Sordaria fimicola*. Lilly and Leonian further showed that the vitamins in question were present in greater concentration near the surface than at lower depths. Müller (29) as cited by Schopfer (40) likewise found various forest and field soils to contain thiamin, the largest amounts being near the surface.

Carpenter (5) observed the presence of riboflavin in a variety of soils and considered that its occurrence was correlated with the amount of organic matter. It was suggested that the presence of the vitamin might be ascribed to the breakdown of plant tissue or to synthesis by soil fungi. More precise quantitative findings were reported by Roulet (35) in 1948. Thiamin was found at a concentration of 19.3 μg per g of soil at a depth of 10 cm, decreasing to 2.9 μg per g at 50 cm, while corresponding amounts for biotin were 0.62 and 0.23 μg per g of soil. Seasonal variation was also noted, chiefly in the surface layers of soil. Roulet (35) and Roulet and Schopfer (36) also reported the detection, in addition to thiamin and biotin, or riboflavin, inositol, pyridoxine, nicotinic acid, and *p*-aminobenzoic acid and ascribed the occurrence of vitamins to synthesis by auxoautotrophic microorganisms, excretory and decomposition products of animals and plants, and to addition of organic manures.

The observation that certain vitamins could be excreted by the roots of certain plants was reported by West in 1939 (45). Flax seedlings grown under sterile conditions were found to liberate measureable amounts of thiamin and biotin. It was inferred that in the soil the development of vitamin-requiring organisms of the rhizosphere depended upon the liberation of such substances by growing plants. While this may be a factor, later information supports the view that their occurrence depends chiefly upon the synthetic abilities of microorganisms.

In 1940 West and Lochhead (46) observed

that many indigenous soil bacteria required one or more preformed vitamins in that they showed growth only in the presence of a combination of thiamin, biotin, nicotinic acid, and inositol. From these preliminary observations Lochhead and Chase (24) developed a scheme for the classification of soil bacteria according to their nutritional requirements and noted in all soils examined a group of organisms which grew only in the presence of a combination of seven vitamins. Furthermore there was evidence of a well-defined group dependent upon a factor or factors present in aqueous extracts of soil. Some of these organisms showed no growth, others at best only slight growth in otherwise suitable media containing inorganic salts and carbohydrates supplemented by combinations of amino acids and vitamins (not including B_{12}) or even yeast extract, but developed well upon the addition of soil extract. It was further noted that for certain strains the nutritive effect of soil extract could be replaced by metabolic filtrates of certain soil bacteria of simple growth requirements, grown in a medium without vitamin supplement; for other strains requiring soil extract such filtrates were ineffective. The findings thus pointed to the presence in soil of more than one unknown growth-promoting substance (23). Following the discovery of vitamin B_{12} and the recognition of its importance in the nutrition of certain microorganisms, Lochhead and Thexton (25, 26) found that for an important proportion, though not all, of the bacteria for which soil extract had previously been considered essential the growth-promoting effect of the latter could be replaced by B_{12} . This vitamin has been detected in soils by assay procedures involving the use of various test organisms; concentrations of 2 to 15 μg per g have been reported in garden, greenhouse, and lawn soils (34) and approximately 2 μg per g in field soils (19, 26).

Another growth factor in soil is represented by the terregens factor (TF) reported by Lochhead and Burton (17), Burton and Lochhead (3), and Burton *et al.* (4). It was found to be required by a group of exacting organisms, typified by *Arthrobacter terregens*, following a study of numerous isolates which required soil extract, but for which none of the known vitamins or yeast extract was able to promote growth. Culture filtrates of certain soil bacteria, *e.g.*, *Arthrobacter pascens* having very simple nutri-

tional requirements were found to replace soil extract for the growth of *A. terreus* which shows response to TF concentrates at approximately 1 μg per ml. Assays of the factor in soil have shown values of about 150 μg per g (19).

III. SYNTHETIC AND DESTRUCTIVE PROCESSES

A notable advance in our knowledge of the relationship of soil microorganisms to the vitamin supply of the soil resulted from the work of Starkey and Schmidt who approached the problem from the standpoint of soil dynamics. Studying the changes in the content of riboflavin, pantothenic acid, and nicotinic acid during the course of decomposition of various organic materials, Starkey (41, 42) noted a general pattern of fluctuation in amounts as decomposition progressed, intimately concerned with the synthetic and destructive action of microorganisms. In straw, grass, and straw-grass mixtures the content of vitamins increased with the rapid initial microbial development; however, as decomposition of the organic matter progressed, the vitamin content markedly decreased. In the later stages the amounts present tended to approach similar levels, irrespective of the initial vitamin contents of the materials. These studies suggested the nature of the changes which occur during the breakdown of organic matter incorporated in soil.

With regard to soil itself, though it has been often stated that the production of vitamins by microorganisms represents an important source of supply, little direct evidence was available until the work of Schmidt and Starkey (39) who determined the amounts of riboflavin and pantothenic acid present during the course of decomposition of plant residues incorporated in soil. As with previous compost experiments, microbial activity resulted initially in marked increases followed by decreases in the content of the vitamins. Pure synthetic riboflavin or pantothenic acid added to soil at rates up to 800 μg per 100 g decreased rapidly. These studies emphasized that soil microorganisms play a highly important role in both the synthesis of vitamins and their decomposition and one may conclude that the concentration of a growth factor at any time depends upon the balance established between synthetic and destructive agencies.

IV. VITAMIN-REQUIRING BACTERIA IN SOIL

In view of the occurrence of B-vitamins in soil it is not surprising that bacteria are present for which specific vitamins are essential for growth. While earlier reports (*e.g.*, 24, 46) established that soil contains groups of microorganisms requiring a combination of known vitamins as well as growth-promoting substances then unidentified, our knowledge of the direct relationship between soil microorganisms and growth factors remained fragmentary and it was not until recently that information became available concerning the abundance in soil of organisms requiring specific growth factors.

A. Basic Procedures

It is self-evident that any detailed study of the qualitative nature of the indigenous bacteria of the soil should be carried out on cultures isolated from as nonselective a medium as possible. Furthermore, the quantitative aspects of any procedure for the classification of such cultures can only be evaluated by the use of non-selective isolation procedures involving large numbers of cultures.

In this laboratory, soil extract agar without added energy material has been used in preference to other media which, though synthetic and hence more easily reproducible, were regarded as more selective (20). Even media based on soil extract but "enriched" with carbohydrates, available nitrogen sources, or yeast extract were found less satisfactory for obtaining isolates representative of the indigenous soil microflora. Any modification of soil extract was considered to increase the selectivity of the medium. On plain soil extract agar, which is not a rich medium, colonies are in general small with little tendency to spread. Antagonistic and associative effects, evident on enriched media, are kept at a minimum. Following an adequately long incubation period, 14 days, counts are normally higher than on the richer substrates (44). To avoid haphazard selection or assumption of identity, all colonies on suitable plates are picked until 100 to several hundred are taken and inoculated into soil extract yeast semisolid agar to serve as stock cultures.

B. Bacteria Requiring Vitamin B₁₂

The first quantitative data on the incidence of soil bacteria requiring a definite growth factor

were provided by Lochhead and Thexton (25, 26). Of 534 strains, isolated from a field soil on a non-selective basis, 41 or 7.6 per cent required vitamin B₁₂ as essential nutrilitite. None of these strains was able to develop in an otherwise suitable medium which included yeast extract although all grew well upon the addition of an extract of soil or cyanocobalamine (2 mμg/ml). However, they were able to develop upon the addition of culture filtrates of certain other soil bacteria, having simple nutritional requirements, in proportion to the amount of B₁₂-active substance synthesized by them.

In a detailed study (18) of the physiological and morphological characteristics of members of this group of B₁₂-requiring organisms, 10 types were recognized. In addition to B₁₂, thiamin was essential for all, and biotin for 8 of the 10 forms. Three of the organisms were observed to be cocci and one a pleomorphic, curved, unbranched filamentous form producing cocci in older cultures. The remaining 6 types, representing 80 per cent of the isolates studied, were considered to belong to the genus *Arthrobacter*. In their lack of response to thymidine or methionine, the organisms appeared to show a high degree of specificity in their requirement for vitamin B₁₂, believed attributable to the highly selective procedure employed in their nutritional differentiation. In a subsequent study of the nature of the vitamin B₁₂ requirement of 19 of these isolates, Ford and Hutner (10) reported only one to respond to Factor A or B or pseudovitamin B₁₂, whereas Goldberg *et al.* (12), in a detailed study of one of these strains, found its response limited to cyanocobalamine and vitamin B₁₂ III.

In a study of three control field soils and rhizosphere soils of three crop plants, namely wheat, barley, and soy beans, Lochhead and Burton (19) examined, for B₁₂ requirement, 2877 cultures isolated by standard nonselective procedures. B₁₂-requiring bacteria comprised, respectively, 6.4, 7.8, and 7.0 per cent of the population of the control soils, corresponding to 4.3, 4.1, and 3.9 millions per g of soil on the basis of the total plate counts. In addition to these forms for which B₁₂ was essential, a roughly equivalent number of organisms were stimulated by the same vitamin. The findings thus pointed to the soil as an important habitat of vitamin B₁₂-requiring organisms earlier considered to be chiefly lactobacilli. In the rhizosphere (zone of influence of the plant

roots) bacteria requiring B₁₂ were proportionately reduced, comprising, for the three crops, 3.7, 2.7, and 3.8 per cent, respectively; however, in view of the pronounced increase in total numbers adjacent to the roots, their absolute numbers were higher than in control soil.

C. Bacteria Requiring the Terregens Factor

Arthrobacter terregens (17), an organism found to require a previously unknown growth-promoting substance present in extracts of soil, is typical of a group of fastidious soil bacteria for which the terregens factor is essential to development. The ability of other soil bacteria, *e.g.*, *Arthrobacter pascens*, to synthesize this factor provides an example of the dependence of certain groups of organisms upon essential substances elaborated by others. Recently Burton (1) compared a number of organisms responding to TF with *A. terregens* and found that they could be grouped into four types on the basis of their physiological characteristics and nutritional requirements. All organisms were alike in responding, not only to TF, but also to certain substances that contain iron, namely coprogen (13) and ferriochrome (30, 31), while a weak response was shown to hemin. In addition to TF they required thiamin and biotin whereas one form showed a need for *p*-aminobenzoic acid, partially replaceable by folic acid. For *A. terregens*, pantothenic acid had been previously found to be essential (3). It is of taxonomic interest that all organisms requiring TF that have been examined belong to the genus *Arthrobacter*. The life cycles of *A. terregens* as well as of *A. pascens*, which synthesizes the factor, have been studied in detail by Chaplin (6).

In a study of the incidence of bacteria requiring TF in soils (19), based on tests with 2877 isolates, it was found that they were roughly one-tenth as numerous as those requiring vitamin B₁₂, with numbers ranging between 100,000 and 500,000 per g of field soil samples. As in the case of those dependent upon B₁₂, organisms requiring TF were proportionately less abundant in the rhizospheres of the crops studied.

D. Specific Vitamin Requirements of the Predominant Bacterial Flora

In an extension of studies on the incidence in soil of bacteria requiring growth factors to include the various vitamins of the B group, Lochhead

TABLE 1
Incidence in a field soil of bacteria requiring specific vitamins

Vitamin Required (Either Alone or with Others)	Vitamin-Requiring Bacteria	
	Percentage of total isolates	Approx no. (millions/g)
Thiamin.....	19.4	10.2
Biotin.....	16.4	8.6
Vitamin B ₁₂	7.2	3.8
Pantothenic acid.....	4.6	2.4
Folic acid.....	3.0	1.6
Nicotinic acid.....	2.0	1.1
Riboflavin.....	0.6	0.3
Pyridoxine.....	<0.2	<0.1
<i>p</i> -Aminobenzoic acid.....	<0.2	<0.1
Choline.....	<0.2	<0.1
Inositol.....	<0.2	<0.1
One or more factors.....	27.1	14.1

Data summarized from reference 22.

and Burton (21, 22) examined the specific requirements of 499 organisms isolated from a field soil. It was found that an important proportion (27.1 per cent, corresponding to 14.1 millions per g) required one or more vitamins for growth. The vitamins found to be essential, either alone or with others, are shown in table 1, in order of frequency. The finding of thiamin as the most commonly required vitamin provides further evidence of the need for this factor so frequently encountered. Biotin, as expected, proved likewise prominent among the essential factors; however, of special interest was the finding of B₁₂ as the third most frequently needed growth factor, the number of organisms for which it was found essential being estimated at 3.8 millions per g of soil, in close agreement with numbers found previously in other soils (see above).

For none of the isolates was pyridoxine, *p*-aminobenzoic acid, choline, or inositol found to be essential, although in some cases these factors stimulated growth. It was considered likely that bacteria requiring one or more of these factors occur in soil; however, the findings suggested that their incidence, estimated at less than 100,000 per g, is relatively small. With the majority of the strains needing vitamins (64 per cent), more than one was required; only in the case of thiamin, biotin, and riboflavin were organisms found that

needed but a single factor. In all, 16 "patterns" of vitamin requirements were found.

V. SYNTHESIS OF GROWTH FACTORS BY SOIL BACTERIA

The belief that the synthetic abilities of microorganisms is a major factor in contributing to the vitamin supply of the soil was based largely on inference rather than on positive evidence before the studies of Schmidt and Starkey (39) referred to above. However, with respect to the capabilities of the soil microflora for synthesis of specific growth-promoting substances, information remained fragmentary and it was only more recently that any report was available concerning the numerical distribution of forms capable of elaborating growth factors. To be sure various vitamins have been shown to be produced by organisms isolated from soil. Thus in recent years interest in vitamin B₁₂ has stimulated a search for organisms capable of synthesizing this vitamin, and various workers have shown that many microorganisms, chiefly bacteria and actinomycetes, including strains isolated from soil, are able to produce B₁₂-active substances (*cf.* Darken (7)). Other than those on B₁₂ production by species of *Rhizobium* (2, 47), however, such studies have had little relationship to the microbial economy of soils as such, though they have shown the soil to be a rich storehouse of organisms with capacity for synthesizing substances which can be turned to practical account in other directions.

A. Capability of the Predominant Soil Bacteria for Vitamin Synthesis

Recently a study has been reported on the incidence in soil of bacteria able to synthesize various growth factors (16). The capacity for synthesis of thiamin, biotin, riboflavin, vitamin B₁₂, and the terregens factor was determined for 316 cultures, comprising approximately equal numbers isolated by nonselective procedures from a control field soil and the rhizosphere soils of rye and barley crops, respectively. Three culture media were employed for each isolate and the presence of growth factors in the classified culture filtrates was determined by using "detector" organisms isolated from soil and selected on the basis of their specific requirements.

With all soils riboflavin was produced by the highest percentage of isolates, followed by thiamin

TABLE 2
Incidence in soil and crop rhizospheres of bacteria capable of synthesizing various growth factors

Growth Factor Produced	Control Soil		Rye Rhizosphere		Barley Rhizosphere	
	Percentage of total isolates	Approx no. (millions/g)	Percentage of total isolates	Approx no. (millions/g)	Percentage of total isolates	Approx no. (millions/g)
Thiamin.....	35.5	41.1	32.4	374.7	56.7	1487.0
Biotin.....	19.6	22.7	15.2	175.8	32.7	857.6
Riboflavin.....	39.2	45.4	35.2	407.1	67.3	1765.0
Vitamin B ₁₂	29.9	34.6	33.3	385.5	34.6	907.4
Terregens factor.....	22.4	25.9	19.0	218.8	25.0	655.6
One or more factors.....	50.5	58.5	59.0	682.4	81.7	2142.6

Data summarized from reference 16.

and B₁₂, somewhat lower proportions being found capable of synthesis of biotin and TF (table 2). One-half of the isolates from the control soil and rather higher percentages of the rhizosphere isolates formed one or more growth factors. No rhizosphere effect was noted with either crop with respect to the percentage of isolates producing B₁₂ or TF; with barley, although not with rye, the relative incidence of organisms forming riboflavin, thiamin, and biotin was greater than in the control soil. However, in view of the pronounced increase in total numbers of organisms adjacent to the roots, the absolute numbers of bacteria capable of producing growth factors were very much higher in the rhizospheres.

VI. DISCUSSION

The data given in table 2 indicate the potentialities of the indigenous soil bacteria for growth-factor synthesis and do not establish that the organisms necessarily produce them in the soil. However, the findings do indicate that these bacteria are able to produce them under appropriate conditions, and there is no reason for doubting that circumstances may provide suitable conditions in the soil. It is difficult to conceive of a method for evaluating, separately, vitamin synthesis and destruction in a natural soil; although vitamin levels may be estimated by appropriate assay methods, the amounts found represent only balances at a given time between simultaneous, but opposing, processes.

Such opposing processes are exemplified by conditions of microbial association where, for example, the vitamin B₁₂ (26) or TF (17) required

by certain organisms and utilized by them depends for its production upon others capable of synthesizing the factor. The situation is further complicated by the utilization of one or more preformed vitamins by organisms capable of synthesizing others (*cf.* Schmidt (38)) as noted in the course of the studies referred to above (16).

A. Some Analogies between Soil and Aquatic Environments

Many marine and freshwater plankton organisms are known to require vitamins, particularly vitamin B₁₂, and the fundamental studies by Hutner and his co-workers have led to knowledge of the role of B₁₂ in the nutrition of many algae and flagellates which had previously been cultivated in media containing extracts of soil or other natural extracts (*cf.* Ford and Hutner (9); Provasoli and Pintner (33); Droop (8)). The evidence so far obtained indicates that in addition to B₁₂, thiamin and, to a lesser extent, biotin are the vitamins most frequently needed by auxotrophic plankton organisms (33). That the same three vitamins seem likewise to be needed by the largest percentage of auxotrophic bacteria from soil is consistent with the relationships that are emerging with respect to certain nutritional aspects of the ecology of the soil and water environments. It is more than an assumption that the growth of certain marine organisms, which require vitamins and whose development, often "explosive," appears to be correlated with previous heavy rains on land, may depend upon nutrients washed into the sea from the land (*cf.* Hutner *et al.* (14)). Although marine microorganisms, chiefly bacteria, doubtless embrace types capable

of vitamin synthesis, they are probably much less abundant than vitamin-producing soil forms and may be expected to be largely associated with particulate matter. The recent work of Starr (43) on amounts of B₁₂ in detritus from samples extending from offshore ocean waters through an estuarine environment to marshland fed by river water shows a pattern of variation exhibiting certain analogies with soil conditions. The findings are consistent with the view that coastal waters may be enriched by growth factors from the soil, that many marine microorganisms may utilize these factors, and that, as in the soil, organisms in water may act in producing, as well as consuming, vitamins.

B. Growth-Promoting Substances and Soil Problems

It seems well established that bacteria, in soil, which require growth factors, as well as those which are able to synthesize such substances, are not academically interesting rarities, but on the other hand comprise important segments of the indigenous soil microflora. One is therefore justified in believing that growth-promoting substances should be taken into account in any consideration of the microbial status or potentiality of the soil. This seems to be particularly relevant to problems of crop growth and plant health, and thus the growth-factor aspects of the rhizosphere environment, where the interrelationships between the plant, the "normal" soil microflora, and soil-borne plant pathogenic organisms are at their most critical, may assume an importance not heretofore recognized.

The available data show that in the rhizosphere of plants the numbers of organisms requiring growth factors, and even more particularly those with capabilities for the production of growth factors, may attain very high levels. In view of this, there is reason for believing that growth factors warrant far more consideration than has been accorded them in studying mutual effects of plant and soil microflora, and in attempts to solve problems concerned with mechanisms of plant-root infection by pathogenic organisms and disease control.

There are suggestions that, apart from a common "rhizosphere effect," different crops may act in significantly different degrees in affecting the microorganisms in their immediate environment, including those capable of syn-

thesizing particular vitamins; however, we know too little of this and of possible effects of such microorganisms on the plant. Further study of mutual effects could add to our knowledge of questions pertaining to crop sequence, as related not only to growth but to susceptibility to disease.

In his historical survey of root-disease investigation, Garrett (11) has described the failure which has attended attempts to control disease caused by soil-borne pathogens by inoculating soil with antibiotic-producing organisms, a result which, as he points out, might have been forecast from ecological considerations. He points out further that until the mechanisms of infection and control have been elucidated, only empirical methods with their attendant limitations can be applied. It is reasonable to assume that such mechanisms must be studied with an awareness that factors that promote, as well as those that suppress, the growth of microorganisms should be considered.

The occurrence in the soil, and particularly in the rhizosphere, of large numbers of microorganisms which depend upon or which synthesize growth factors points to the need for more than the scattered information we possess concerning the role of vitamins and other growth-promoting substances. More precise knowledge is required of growth-promoting exudates of roots, of fungal spore germination activators, and of factors required or produced by microorganisms, including pathogens, as related to their differential effects on saprophytic and parasitic microorganisms in the soil and on the plant itself. Most of the questions posed by such considerations, however, remain as yet unanswered.

VII. REFERENCES

1. BURTON, M. O. 1957 Characteristics of bacteria requiring the terregens factor. *Can. J. Microbiol.*, **3**, 107-112.
2. BURTON, M. O., AND LOCHHEAD, A. G. 1952 Production of vitamin B₁₂ by *Rhizobium* species. *Can. J. Botany*, **30**, 521-524.
3. BURTON, M. O., AND LOCHHEAD, A. G. 1953 Nutritional requirements of *Arthrobacter terregens*. *Can. J. Botany*, **31**, 145-151.
4. BURTON, M. O., SOWDEN, F. J., AND LOCHHEAD, A. G. 1954 Studies on the isolation and nature of the terregens factor. *Can. J. Biochem. Physiol.*, **32**, 400-406.
5. CARPENTER, C. C. 1943 Riboflavin-vitamin B₂ in soil. *Science*, **98**, 109-110.

6. CHAPLIN, C. E. 1957 Life cycles in *Arthrobacter pascens* and *Arthrobacter terregens*. Can. J. Microbiol., **3**, 103-106.
7. DARKEN, M. A. 1953 Production of vitamin B₁₂ and its occurrence in plant tissue. Botan. Rev., **19**, 99-130.
8. DROOP, M. R. 1957 Auxotrophy and organic compounds in the nutrition of marine phytoplankton. J. Gen. Microbiol., **16**, 286-293.
9. FORD, J. E., AND HUTNER, S. H. 1955 Role of vitamin B₁₂ in the metabolism of microorganisms. Vitamins and Hormones, **13**, 101-136.
10. FORD, J. E., AND HUTNER, S. H. 1957 On the nature of the vitamin B₁₂ requirements in soil bacteria isolated by Lochhead and his co-workers. Can. J. Microbiol., **3**, 319-327.
11. GARRETT, S. D. 1955 A century of root-disease investigation. Ann. Appl. Biol., **42**, 211-219.
12. GOLDBERG, M. K., HUTNER, S. H., AND FORD, J. E. 1957 Nutrition of a cobalamin-requiring soil bacterium. Can. J. Microbiol., **3**, 329-334.
13. HESSELTINE, C. W., PIDACKS, C., WHITEHILL, A. R., BOHONOS, N., HUTCHINGS, B. L., AND WILLIAMS, J. H. 1952 Coprogen, a new growth factor for coprophilic fungi. J. Am. Chem. Soc., **74**, 1362.
14. HUTNER, S. H., PROVASOLI, L., McLAUGHLIN, J. J. A., AND PINTNER, I. J. 1956 Biochemical geography: some aspects of recent vitamin research. Geograph. Rev., **46**, 404-407.
15. LILLY, V. G., AND LEONIAN, L. H. 1939 Vitamin B₁ in soil. Science, **89**, 292.
16. LOCHHEAD, A. G. 1957 Qualitative studies of soil microorganisms. XV. Capability of the predominant bacteria flora for synthesis of various growth factors. Soil Sci., **84**, 395-403.
17. LOCHHEAD, A. G., AND BURTON, M. O. 1953 An essential bacterial growth factor produced by microbial synthesis. Can. J. Botany, **31**, 7-22.
18. LOCHHEAD, A. G., AND BURTON, M. O. 1955 Qualitative studies of soil microorganisms. XII. Characteristics of vitamin B₁₂-requiring bacteria. Can. J. Microbiol., **1**, 319-330.
19. LOCHHEAD, A. G., AND BURTON, M. O. 1956 Incidence in soil of bacteria requiring vitamin B₁₂ and the terregens factor. Soil Sci., **82**, 237-245.
20. LOCHHEAD, A. G., AND BURTON, M. O. 1956 Importance of soil extract in the enumeration and study of soil bacteria. Trans. Intern. Congr. Soil Sci., 6th Congr., Paris, C, 157-161.
21. LOCHHEAD, A. G., AND BURTON, M. O. 1956 Soil as a habitat of vitamin-requiring bacteria. Nature, **178**, 144-145.
22. LOCHHEAD, A. G., AND BURTON, M. O. 1957 Qualitative studies of soil microorganisms. XIV. Specific vitamin requirements of the predominant bacterial flora. Can. J. Microbiol., **3**, 35-42.
23. LOCHHEAD, A. G., AND CHASE, F. E. 1942 Bacterial growth factors in soil. Science, **96**, 387.
24. LOCHHEAD, A. G., AND CHASE, F. E. 1943 Qualitative studies of soil microorganisms. V. Nutritional requirements of the predominant bacterial flora. Soil Sci., **55**, 185-195.
25. LOCHHEAD, A. G., AND THEXTON, R. H. 1951 Vitamin B₁₂ as a growth factor for soil bacteria. Nature, **167**, 1034.
26. LOCHHEAD, A. G., AND THEXTON, R. H. 1952 Qualitative studies of soil microorganisms. X. Bacteria requiring vitamin B₁₂ as growth factor. J. Bacteriol., **63**, 219-226.
27. LWOFF, A., AND LEDERER, E. 1935 Remarques sur l'extrait de terre envisagé comme facteur de croissance pour les flagellés. Compt. rend. soc. biol., **119**, 971-973.
28. MOCKERIDGE, F. A. 1920 The occurrence and nature of the plant growth-promoting substances in various organic manurial composts. Biochem. J., **14**, 432-450.
29. MÜLLER, W. F. 1941 Zur Wirkstoffphysiologie des Bodenpilzes *Mucor ramannianus*. Ber. schweiz. botan. Ges., **51**, 165.
30. NEILANDS, J. B. 1952 A crystalline organo-iron pigment from a smut fungus (*Ustilago sphaerogena*). J. Am. Chem. Soc., **74**, 4846-4847.
31. NEILANDS, J. B. 1957 Some aspects of microbial iron metabolism. Bacteriol. Revs., **21**, 101-111.
32. PRINGSHEIM, E. G. 1935 Wuchsstoffe im Erdboden? Naturwissenschaften, **12**, 197-198. (Ref. Chem. Abs., 1935, **29**, 4866).
33. PROVASOLI, L., AND PINTNER, I. J. 1953 Ecological implications of *in vitro* nutritional requirements of algal flagellates. Ann. N. Y. Acad. Sci., **56**, 839-851.
34. ROBBINS, W. J., HERVEY, A., AND STEBBINS, M. E. 1950 Studies on *Euglena* and vitamin B₁₂. Bull. Torrey Botan. Club., **77**, 423-441.
35. ROULET, M. A. 1948 Recherches sur les vitamines du sol. Experientia, **4**, 149-150.
36. ROULET, M. A., AND SCHOPFER, W. H. 1950

- Les vitamines du sol et leur signification. Trans. Intern. Congr. Soil Sci. 4th Congr., Amsterdam, 1, 202-203.
37. SANBORN, J. R. 1927 Essential food substances in soil. *J. Bacteriol.*, **13**, 113-121.
38. SCHMIDT, E. L. 1951 Soil microorganisms and plant growth substances. I. Historical. *Soil Sci.*, **71**, 129-140.
39. SCHMIDT, E. L., AND STARKEY, R. L. 1951 Soil microorganisms and plant growth substances. II. Transformations of certain B-vitamins in soil. *Soil Sci.*, **71**, 221-231.
40. SCHOPFER, W. H. 1943 *Plants and vitamins*. Chronica Botanica Co., Waltham, Massachusetts.
41. STARKEY, R. L. 1942 Transformation of riboflavin and pantothenic acid during decomposition of plant materials. *Soil Sci. Soc. Am., Proc.*, **7**, 237-242.
42. STARKEY, R. L. 1944 Changes in the content of certain B-vitamins in organic materials decomposing under aerobic and anaerobic conditions. *Soil Sci.*, **57**, 247-270.
43. STARR, T. J. 1956 Relative amounts of vitamin B₁₂ in detritus from oceanic and estuarine environments near Sapelo Island, Georgia. *Ecology*, **37**, 658-664.
44. STEVENSON, I. L., AND ROUATT, J. W. 1953 Qualitative studies of soil microorganisms. XI. Further observations on the nutritional classification of bacteria. *Can. J. Botany*, **31**, 438-447.
45. WEST, P. M. 1939 Excretion of thiamine and biotin by the roots of higher plants. *Nature*, **144**, 1050-1051.
46. WEST, P. M., AND LOCHHEAD, A. G. 1940 Qualitative studies of soil microorganisms IV. The rhizosphere in relation to the nutritive requirements of soil bacteria. *Can. J. Research, C*, **18**, 129-135.
47. VYAS, S. R., AND PRASAD, N. 1956 Production of vitamin B₁₂ by rhizobia in Gujarat soil. *J. Sci. Ind. Research (India)*, **15C**, 52